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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—Circular No. 85.

B. T. GALLOWAY, Chief of Bureau.

CROWN-GALL AND SARCOMA.

By Erwin F. Smith,

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INTRODUCTION.

In a recent bulletin of the Bureau of Plant Industry of the United States Department of Agriculture[1] the writer pointed out resemblances in crown-gall of plants to malignant animal tumors and especially to sarcoma. Since the publication of this bulletin further study has revealed several very interesting facts. Our observations show that in method of development this disease is strikingly like that of certain malignant neoplasms in man, especially those in which a distinct strand of tumor tissue has been traced from the primary tumor to the secondary tumor.

There is perhaps no metastasis, in the strict sense of the term, but so far as regards the mechanism of tumor development it is a matter of minor importance whether the migrating tumor cells separate themselves entirely from the parent mass or remain connected with it by means of a tumor strand. The plant body favors the latter method of development, whereas the rapidly circulating blood stream in animals favors the former, i. e., the dislodgment and distribution of cells. The matter of chief importance is the method of action of the parasite on the cells of the host.

QUESTIONS UNSOLVED.

In the bulletin mentioned the following subjects were either not touched upon or left in an inconclusive state:

(1) The question of the presence or absence of the bacteria in the secondary tumors.

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[1] Bulletin 213, Bureau of Plant Industry, U. S. Dept. of Agriculture, entitled "Crown-Gall of Plants: Its Cause and Remedy." The edition of this bulletin is exhausted at the Department of Agriculture. It may be obtained, however, from the superintendent of documents, Government Printing Office, Washington, D. C. Price 40 cents.

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(2) The relation of the origin of the secondary tumors to the primary tumor, i. e., whether the new growths result from the independent migration of bacteria from the primary tumor to the site of the secondary tumor or whether the tumor cell itself is the migrant. That the latter was believed to be the case may be seen from the second paragraph on page 164.

(3) A comparison of the cell structure of the secondary tumor with that of the primary tumor.

NEW FACTS.

Having obtained much favorable material for study from inoculations made on the daisy in January of this year, using for inoculation pure cultures of the schizomycete plated from tumors on the daisy, the answers to these questions are at hand and are confirmatory of the hypothesis made in the bulletin, viz, that the study of crown-gall is calculated to throw some light on the origin of malignant animal tumors.

This study has yielded the following results.

BACTERIA IN SECONDARY TUMORS.

The bacterium causing the primary tumor occurs also in the secondary tumors. It is not plentiful, but, so far as we have plated, occurs in about the same numbers as in the primary tumor. Numerous poured-plate isolations and several successful reinoculations have put beyond question its normal occurrence in these secondary tumors. It occurs also sparingly in the tissue between tumors. In other words, we do not have in this disease a cell stimulus begun in the primary tumors by a parasite and then able to propagate itself independently of the inciting organism. On the contrary, the bacteria and the tumor cells occur together both in the primary tumor and in the secondary tumor *and the one inside the other.* Owing to our inability thus far to stain the causal organism inside the cells without at the same time staining many confusing granules or to see it therein under the microscope so as to determine its presence in unstained sections with certainty, the evidence for this last statement is indirect, but none the less conclusive. This evidence is twofold: (a) By the poured-plate method, from the interior of tumor tissue after surface sterilization we obtain the tumor-producing schizomycete, and, therefore, it occurs in these tissues; (b) when such tissues are sectioned and studied under the microscope the vessels and the intercellular spaces, so far as we have been able to observe, are free of bacteria. Numerous observations made during the last three months confirm many earlier ones. If bacteria were commonly present in the vessels or spaces between the cells (as happens

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in many other plant diseases) they ought to be readily visible either stained or unstained, for, quite unlike the cells, these spaces are free of all confusing granular matters. By the doctrine of exclusion, therefore, the bacteria must be within the cells. The discovery of them in place is complicated by the fact that they are not very numerous, are not acid fast, are probably often in the form of involution cells or in some other form difficult to identify, and are mixed in with numerous minute crystals, crystalloids, and other granules of the host protoplasm, which itself takes bacterial stains readily.

TUMOR STRANDS.

In many instances (we can not yet affirm in all) the secondary tumors are connected with the primary tumor by a deep-seated strand or pedicel of tumor tissue. This strand wedges its way through the interior of stems and leaves, *after the manner of a foreign body*, until in some suitable place, often at a considerable distance from the primary tumor, it gives rise to a deep secondary tumor, which subsequently ruptures through to the surface of the plant. This offshoot from the primary tumor occurs deep in the wood, at the junction of wood and pith, and may sometimes be seen with the naked eye, owing to the fact that the chloroplasts in its cells become more numerous than elsewhere and give to the strand a deep-green color and because being under great pressure it wells out on cross section of the stem, something like a cut muscle; at other times the strand is restricted to a few cells and can be made out with difficulty even under the microscope, but wherever it occurs in any quantity it is seen to be totally distinct in its structure from the normal tissues surrounding it, conforming to the tumor tissue in its histology, and can be traced in the one direction to the primary tumor and in the other to the secondary, and frequently in the course of its progress from the primary to the secondary tumor we have observed island-like enlargements, and comparison of these in various stages of development show that they are the beginnings of other tumors. Sometimes these enlargements of the green strand have progressed far enough to push up the normal tissues and show as a swelling on the outside of the stem; at other times not. Finally, from this strand we have cultivated out *Bacterium tumefaciens*, and reproduced the disease with the cultures. Frequently the wood in this part of the stem takes on an increased growth.

SECONDARY TUMORS HAVE THE STRUCTURE OF THE PARENT TUMOR.

As might be expected from statements under the previous heading, "Tumor strands," when the primary tumor occurs on the stem and secondary tumors subsequently appear in the leaves the structure of the leaf tumors is approximately that of the stem. We have, then, in place of the leaf traces and loose cellular structure of the ordinary dorsiventral petiole or midrib, a firm structure consisting of a greenish pseudopith (of tumor tissue), surrounded by a cylinder of wood, beyond which is a cambium and then a bark covered with cork. This stem structure is sometimes very perfect, although less woody than a normal stem—i. e., of a looser structure—at other times, incomplete on one side, only a partial cylinder of wood being formed. This stem structure may develop either in the central leaf trace or in one or more of the side leaf traces, according as one or the other is penetrated by the advancing strand of tumor tissue. All stages of this conversion have been seen, from petioles which still present their external form and show only a slight disturbance in one of the leaf traces to those in which all resemblance to an ordinary leaf has disappeared, the petiole being displaced by a thick, firm, stemlike growth more or less circular in cross section and totally unlike the dorsiventral structure of a leaf. Even in such cases traces of the unchanged wings of the petiole may be seen sometimes as marginal appendages of the secondary tumor.

Full details will be given in another bulletin as soon as the illustrations can be prepared.

Approved:
JAMES WILSON,
Secretary of Agriculture.

WASHINGTON, D. C., *June 17, 1911.*
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